

Claims 20-23 (cancelled).

Listing of Claims:

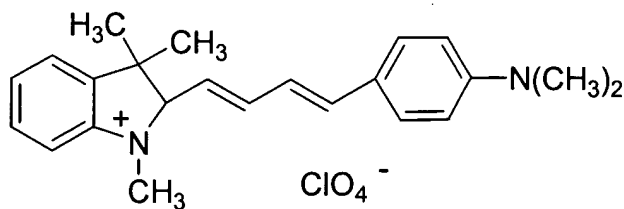
1. (Currently Amended) A method of staining bacteria in a sample comprising: adding ~~working~~ a polymethine dye to the ~~on a~~ sample in the presence of a substance capable of reducing nitrite ions ~~to stain bacteria in the sample~~.

2. (Original) A method according to claim 1, wherein the substance capable of reducing nitrite ions is selected from the group consisting of: ascorbic acid, isoascorbic acid, aminomethanesulfonic acid, aminoethanesulfonic acid, glutamic acid, asparatic acid, mercaptoacetic acid, 3-mercaptopropionic acid, sulfamic acid, sulfanilic acid, sulfurous acid, pyrosulfurous acid, phosphinic acid, glycine, glutamine, asparagine, methionine, glutathione, cysteine, hydroxylamine and salts thereof; sulfanilamide; aminomethane; mercaptoethanol; thiophenol and urea.

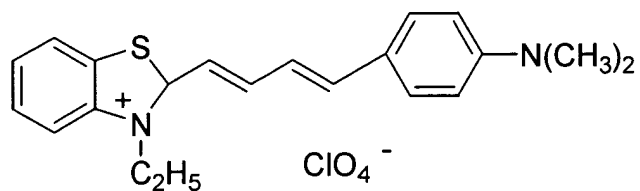
3. (Currently Amended) A method according to claim 1, wherein the polymethine dye is at least one selected from the following group consisting of:

(1) Thiazole Orange;

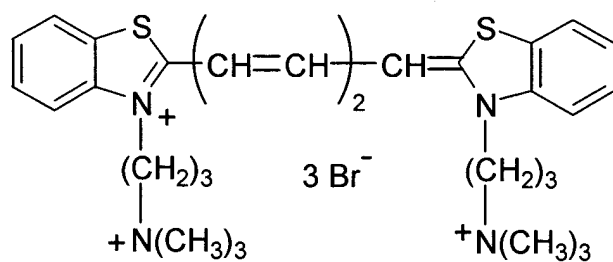
(2)



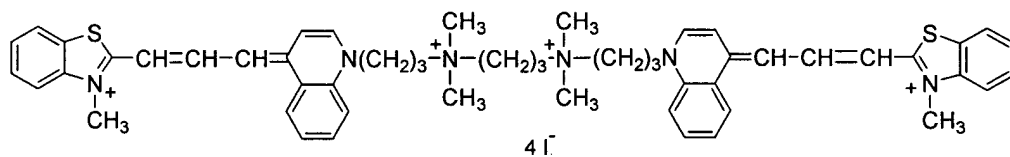
(3)



(4)

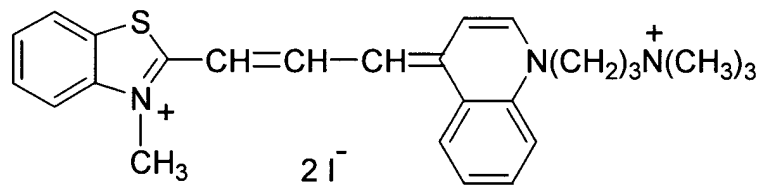


(5)



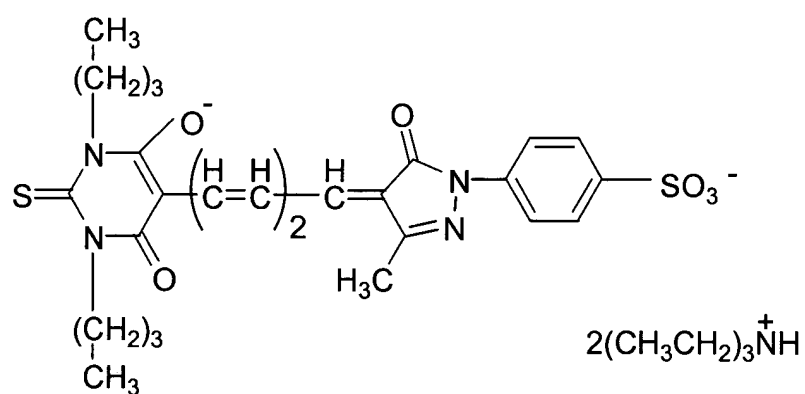
[(5)]

(6)

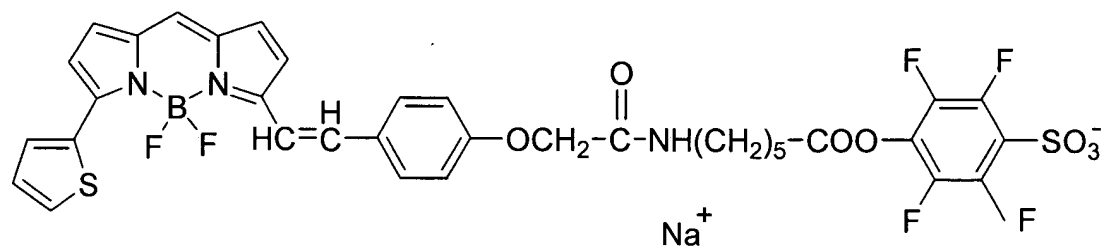


[(6)]

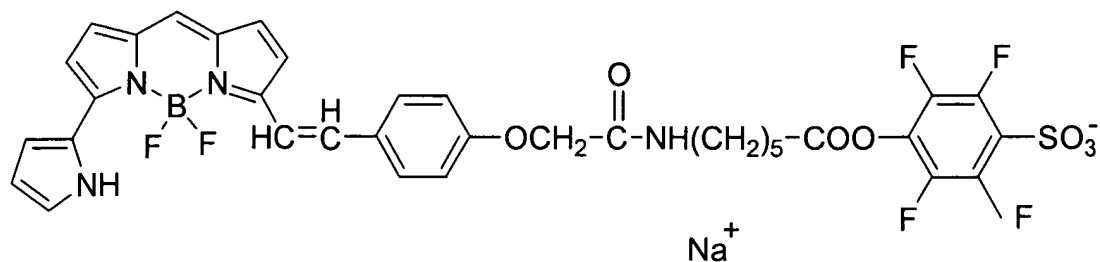
(7)



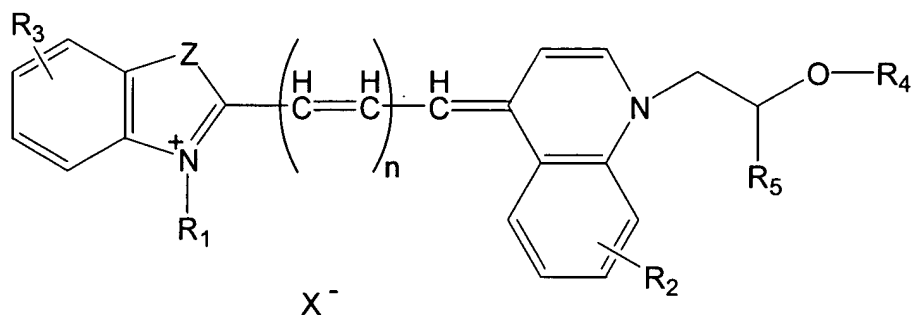
(8)



(9)

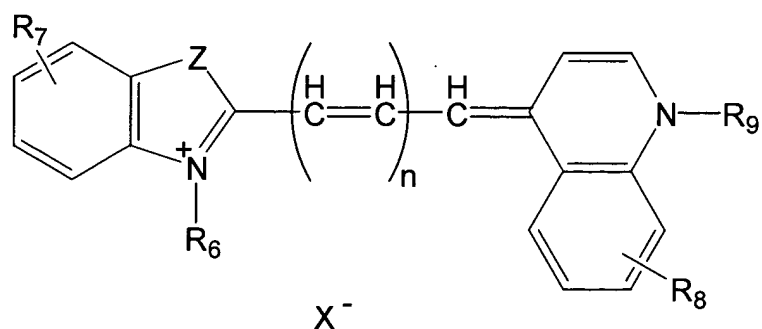


(10) a compound represented by the following general formula:



wherein R_1 is a hydrogen atom or a C_{1-3} alkyl group; R_2 and R_3 are a hydrogen atom, a C_{1-3} alkyl group or a C_{1-3} alkoxy group; R_4 is a hydrogen atom, an acyl group or a C_{1-3} alkyl group; R_5 is a hydrogen atom or a C_{1-3} alkyl group which may be substituted; Z is a sulfur atom, an oxygen atom or a carbon atom substituted with a C_{1-3} alkyl group; n is 1 or 2; X^- is an anion; and

(11) a compound represented by the following general formula:

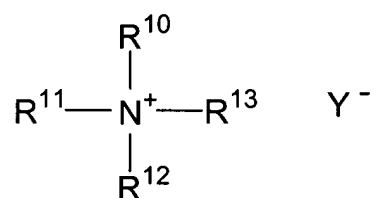


(11) a compound represented by the following general formula:

wherein R₁ is a hydrogen atom or a C₁₋₁₈ alkyl group; R₂ and R₃ are a hydrogen atom, a C₁₋₃ alkyl group or a C₁₋₃ alkoxy group; R₄ is a hydrogen atom, an acyl group or a C₁₋₁₈ alkyl group; Z is sulfur, oxygen or carbon having a C₁₋₃ alkyl group; n is 0, 1 or 2; ~~X~~ X⁻ is an anion.

4. (Currently Amended) A method according to claim 1, ~~wherein the working is carried out in the existence with~~ presence of a cationic surfactant.

5. (Currently Amended) A method according to claim 4, wherein the cationic surfactant is a quaternary ammonium salt represented by the following formula:



wherein R¹⁰ is a C₆₋₁₈ alkyl group or (C₆H₅)-CH₂-; R¹¹, R¹² and R¹³, the same or different, are a C₁₋₃ alkyl group or a benzyl group; ~~Y~~ Y⁻ is a halogen ion.

6. (Original) A method according to claim 5, wherein the quaternary ammonium salt is at least one selected from the group consisting of: decyl trimethyl ammonium salt, dodecyl trimethyl ammonium salt, tetradecyl trimethyl ammonium salt, hexadecyl trimethyl ammonium salt and octadecyl trimethyl ammonium salt.

7. (Currently Amended) A method according to claim 1, wherein the ~~dye is worked under~~ sample is in an acidic state.

8. (Original) A method according to claim 7, wherein the acidic state is set at pH 2.0-4.5.

9. (Currently Amended) A method according to claim 4 7, wherein a buffer of pKa 1-5.5 is used to maintain an acidic pH.

10. (Original) A method according to claim 9, wherein the buffer is at least one selected from the group consisting of: citric acid-NaOH, potassium dihydrogen phosphate-disodium hydrogen phosphate, potassium dihydrogen phosphate-NaOH, citric acid- disodium hydrogen phosphate, potassium hydrogen phthalate-NaOH, succinic acid-NaOH, lactic acid-NaOH, ε-aminocaproic acid-HCl, fumaric acid-HCl, β-alanine-NaOH and glycine-NaOH.

11. (Currently Amended) A method according to claim 1, ~~wherein the working is carried out in the existence with~~ presence of an inorganic salt of either sulfate or nitrate.

12. (Currently Amended) A method according to claim 1, wherein the dye is present in an amount of ~~worked at~~ 0.1 to 100 ppm in the sample.

13. (Currently Amended) A method according to claim 1, wherein the substance capable of reducing nitrite ions exists in the sample in such an amount that it can reduce[s] the nitrite ions produced by bacteria by ~~of~~ 10^5 to 10^8 /ml.

14. (Currently Amended) A method according to claim 4 ~~4~~, wherein the cationic surfactant exists at 10 to 30000 mg/l in the sample.

15. (Currently Amended) A method according to claim 10, wherein the ~~acid or the compound~~ buffer maintaining an acidic pH exists at 10 to 500 mM in the sample.

16. (Original) A method according to claim 1, wherein the sample is a urine, blood or spinal fluid.

17. (Currently Amended) The method according to claim 1, further comprising the steps of A ~~method of detecting and counting bacteria comprising the following steps of:~~

~~————— (1) working a polymethine dye on a sample by a method as described in any one of claims 1 to stain bacteria in the sample,~~

~~(2) (1) introducing the thus-treated sample containing the stained bacteria into a detecting part of a flow cytometer and irradiating cells of the stained bacteria one-by-one with light to measure scattered light and fluorescent light emitted from each of the cells the stained bacteria; and~~

~~(3) (2) discriminating the bacteria from other components in accordance with an intensity of a scattered light signal and an intensity of a fluorescent light signal or a pulse width reflecting the length of particles to counting the number of the stained bacteria.~~

18. (Currently Amended) A method according to claim 17, wherein the step of staining the bacteria (1) is carried out in the presence of ~~by the steps of~~

~~—— (a) mixing a sample with an aqueous solution containing a substance capable of reducing nitrite ions and/or a cationic surfactant [to accelerate dye transmissivity of bacteria;~~

~~—— (b) staining the bacteria for a predetermined period with a polymethine dye;~~

19. (Currently Amended) A method according to claim 17, wherein the step of (3) of discriminating and counting the stained bacteria is carried out by determining the parameters selected from the group consisting of ~~in accordance with at least one selected from the following combinations of:~~

(i) a forward scattered light intensity and a forward scattered light pulse width;

(ii) a forward scattered light intensity and a fluorescent light intensity; and

(iii) a forward scattered light pulse width and a fluorescent light intensity.

20. (Canceled)

21. (Canceled)

22. (Cancelled)

23. (Canceled)